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A randomized placebo-controlled study

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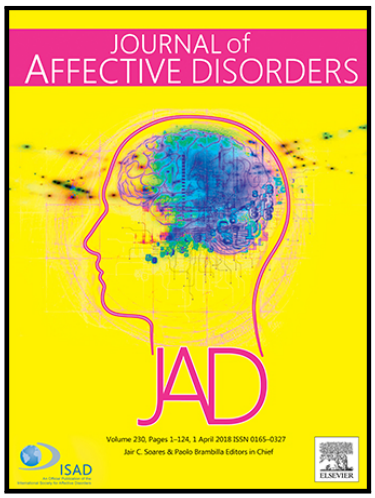

The efficacy of adjunctive N-acetylcysteine in acute bipolar depression: a randomized placebo-controlled study

Pernille Kempel Ellegaard , Rasmus Wentzer Licht , René Ernst Nielsen , Olivia May Dean , Michael Berk , Henrik Enghusen Poulsen , Mohammadreza Mohebbi , Connie Thuroee Nielsen

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HIGHLIGHTS

- The study did not show a statistically significant difference based on the Montgomery-Asberg Depression Rating Scale (MADRS) score after 20 weeks of adjunctive N-acetylcysteine (NAC) treatment compared to placebo
- The MADRS score was reduced statistically significantly from baseline to week 20 in both study groups, and further reduced in the NAC study group at week 24
- The mania score increased in the NAC treated group as compared to the placebo group

The efficacy of adjunctive N-acetylcysteine in acute bipolar depression: a randomized placebo-controlled study

Running head: Add-on N-acetylcysteine for Bipolar Depression

First author: PhD Pernille Kempel Ellegaard[1,2,3*]
Second author: Professor, PhD, MD Rasmus Wentzer Licht[4,5]
Third author: Associate professor, PhD, MD René Ernst Nielsen[4,5]
Fourth author: PhD, Dr Olivia May Dean[6,7,8]
Fifth author: Professor, MBBS Michael Berk[6,7,8,9]
Sixth author: Professor, DMSci Henrik Enghusen Poulsen[10,11]
Seventh author: PhD, Dr Mohammadreza Mohebbi[12]
Eight author: PhD, MD Connie Thuroee Nielsen[1,13]

Affiliations:
[1] Institute of Regional Health Services Research, Faculty of Health Sciences, University of Southern Denmark;
[2] Research Unit, Mental Health Service Esbjerg, The Region of Southern Denmark, Denmark;
[3] OPEN, Odense Patient Data Explorative Network, Odense University Hospital/Institute of Clinical Research, University of Southern Denmark, Odense C, Denmark;
[4] Unit for Psychiatric Research, Psychiatry, Aalborg University Hospital, Denmark;
[5] Department of Clinical Medicine, Aalborg University, Aalborg, Denmark;
[6] Deakin University, IMPACT Strategic Research Centre, School of Medicine, Barwon Health, Geelong, Australia;
[7] Florey Institute for Neuroscience and Mental Health, University of Melbourne, Parkville, Australia;
[8] University of Melbourne, Department of Psychiatry, Royal Melbourne Hospital, Parkville, Australia;
[9] Orygen, The National Centre of Excellence in Youth Mental Health, Parkville, Victoria, Australia
[10] Institute of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark;
[11] Clinical Pharmacology, Bispebjerg Frederiksberg Hospital, Denmark;
[12] Biostatistics Unit, Faculty of Health, Deakin University, Geelong, Australia;
[13] Mental Health Service Vejle, The Region of Southern Denmark, Denmark;

Corresponding author:
Pernille Kempel Ellegaard,
Research Unit, Mental Health Service Esbjerg, Gl. Vardevej 101, 6715 Esbjerg N, Denmark;
Telephone: +45 99 44 71 57. E-mail: Pernille.Ellegaard@rsyd.dk

Abstract

Objective: To investigate the efficacy of adjunctive N-acetylcysteine (NAC) for the treatment of acute bipolar depression.

Method: A randomized, double-blind, multicentre, placebo-controlled trial including adult subjects diagnosed with bipolar disorder, currently experiencing a depressive episode. Participants were treated with 3 g/day NAC or placebo as an adjunctive to standard treatment for 20 weeks, followed by a 4-week washout where the blinding was maintained. The primary outcome was the mean change in the Montgomery Asberg Depression Rating Scale (MADRS) score over the 20-week treatment phase. Linear Mixed Effects Repeated Measures (LMERM) was used for analysing the primary outcome.

Results: A total of 80 subjects were included. The mean MADRS score at baseline was 30.1 and 28.8 in participants randomized to NAC and placebo, respectively. Regarding the primary outcome measure, the between-group difference (NAC vs. placebo) was 0.5, which was statistically non-significant (95% CI: -7.0 - 5.9; p= 0.88). All findings regarding secondary outcomes were statistically or clinically insignificant.

Limitations: The study had a placebo response rate of 55.6% - high placebo response rates are associated with failure to separate from placebo.

Conclusions: Based on our primary outcome measure, we could not confirm previous studies showing a therapeutic effect of adjunctive NAC treatment on acute bipolar depression. Further studies with larger samples are needed to elucidate if specific subgroups could benefit from adjunctive NAC treatment.

KEYWORDS: Bipolar disorder, depression, N acetylcysteine, glutathione, treatment

HIGHLIGHTS

- The study did not show a statistically significant difference based on the Montgomery-Asberg Depression Rating Scale (MADRS) score after 20 weeks of adjunctive N-acetylcysteine (NAC) treatment compared to placebo
- The MADRS score was reduced statistically significantly from baseline to week 20 in both

study groups, and further reduced in the NAC study group at week 24

- The mania score decreased in the NAC treated group as compared to the placebo group

INTRODUCTION

Bipolar depression is challenging to treat, partly due to few available treatments (Grunze et al., 2013). Therefore, search for new treatment options are warranted, and over the recent years, N-acetylcysteine (NAC) has been examined in bipolar disorder (BD) and other mental and neuropsychiatric disorders (Berk et al., 2013; Dean et al., 2011; Deepmala et al., 2016; Minarini et al., 2017). Additionally, NAC has been used in other areas of medicine for almost four decades (D. and Conner, 1978) and is currently used for treating paracetamol overdose (Dringen and Hirrlinger, 2003), chronic obstructive pulmonary disease (Atkuri et al., 2007) and Human Immunodeficiency Virus-infection (Dodd et al., 2008).

NAC has several mechanisms of action that make it potentially useful for the treatment of bipolar depression. Thus, NAC reduces oxidative stress (predominantly though the glutathione (GSH) system), and has also demonstrated anti-inflammatory properties (Zafarullah et al., 2003). BD is associated with an increased systemic inflammation, likely contributing to the pathophysiology (Berk et al., 2013), as it is believed that altered brain-derived neurotrophic factor and inflammatory cytokines are linked to cognitive impairment and neurodegeneration in the disorder (Duman and Monteggia, 2006; Hashimoto et al., 2004; Kim et al., 2007). Additionally, NAC modulates glutamate (Himi et al., 2003; Janaky et al., 2007), which has an impact on cognitive processing and mood states (Berk et al., 2007; Hashimoto et al., 2007). Previous Australian studies have demonstrated efficacy of adjunctive NAC for bipolar depression (Berk et al., 2008; Berk et al., 2011). Thus, NAC 2 g/day used as an add-on to treatment as usual was shown to reduce the Montgomery-Asberg Depression Rating Scale (MADRS) score in a 24-weeks double blinded clinical trial as compared to placebo add-on(Berk et al., 2008). A similar result was found in an 8-week open clinical trial using adjunctive NAC 2 g/day treatment (Berk et al., 2011). However, a 20-weeks double blind maintenance study demonstrated no between-group differences between adjunctive NAC treatment and placebo, possibly due to all study participants receiving open label adjunctive NAC treatment the first eight weeks prior to randomization (Berk et al., 2012).

Aims of the Study

In the current study, we aimed at testing whether the previous positive findings could be replicated in a Danish study sample, by comparing the efficacy of NAC 3 g/day as add-on to treatment as usual compared with placebo add-on, in reducing symptoms of depression in subjects with bipolar depression, and whether the higher dose of NAC chosen here might even increase the magnitude of efficacy. The selected dose of NAC treatment has been reported to be well-tolerated (Prado et al., 2015; Sarris et al., 2015; Schmaal et al., 2011).

MATERIAL AND METHODS

Design

The NACOS-study was a 20-week randomized, double-blind, placebo-controlled, parallel-group, multicentre trial with a 4-week wash-out period, with blinding upheld until last patient had had last visit. The subjects were informed that the last four weeks was a wash-out period where no study medication was provided. The study protocol is published elsewhere(Ellegaard et al., 2018).

After informed consent was signed, the study participants were randomly allocated to NAC or placebo add-on according to a pre-constructed computer-generated randomization list divided into blocks of eight. There was no specific number of blocks allocated to study centre 1 and 2, however study centre 3 was allocated to one and a half block (Centre 1: Esbjerg, Varde and Broerup; centre 2: Fredericia, Vejle and Kolding; centre 3: Aalborg). Consecutively enrolled participants in each study centre received a randomization number at entry into the trial. Study visits were planned at week two, week 10, week 20 and week 24 (± seven days for each visit). Furthermore, participants were contacted via telephone six and 15 weeks after inclusion (± seven days) to support adherence. Un-blinding during the study was not necessary, but Glostrup Pharmacy could be contacted 24 hours a day, if un-blinding was necessary. The Mental Health Services facilities from three study centres covered three catchment areas; the centres were hospital based psychiatric settings, comprising both inpatient and outpatient units. The study procedures were conducted in the Mental Health Service clinics or alternatively as a home visits, whichever was most suitable for the participants.

Ethics

The trial was conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. The Good Clinical Practice Unit at Odense University Hospital and Aalborg Hospital monitored the study. The trial was approved by The Regional Scientific Ethical Committees for Southern Denmark: 35664-20120177. The study was registered at ClinicalTrials.Gov (NCT02294591), and the European Clinical Trials Database (EudraCT: 2012-004483-22).

Treatment

Participants were randomized to 3 grams of NAC/day (1,500 grams (three capsules) twice daily) or placebo tablets identical in appearance, for 20 weeks in addition to Treatment-As-Usual (TAU) followed by four weeks washout, where only TAU was given. Standard mental care treatments including adjustment of psychopharmacological treatment or psychotherapy, initiation or discontinuation of ECT treatment were allowed throughout the study period. TAU was conducted by the clinical staff at the units where the patients were followed.

Eligibility criteria and study withdrawal

Inclusion criteria: 1) age from 18-64 years (both inclusive); 2) fulfilment of DSM-IV criteria for a BD (type I or II) (American Psychiatric Association, 2000), with a current depressive episode; 3) duration of episode at least four weeks; 4) a Montgomery-Asberg Depression Rating Scale score (MADRS) (Montgomery and Åsberg, 1979) score at entry of ≥ 18; 5) at least one documented affective episode within the last six months within the index period (depressive, manic or mixed), as judged by the principal investigator (according to the medical journal, and clinical interview based on the M.I.N.I), 6) written informed consent obtained; 7) female participants had to undergo a pregnancy test at baseline, and were required to be using a safe form of contraception (defined as birth control pills, mini-pills, intrauterine device or birth control implants), and accept that their male partners should use a condom.

Diagnosis was confirmed by the use of the Mini International Neuropsychiatric Interview (M.I.N.I., version 5.0 in Danish) (Amorim et al., 1998; Lecrubier et al., 1998; Sheehan et al.).

Exclusion criteria: 1) received electroconvulsive therapy (ECT) within the last four weeks prior to inclusion; 2) having a daily intake of more than 500 mg NAC, 200 µm selenium, or 500 IU of

vitamin E prior to inclusion; 3) recent bleeding in respiratory organs, asthma, epilepsy or any other medical condition that could interfere with study outcomes, as judged by an investigator; 4) allergy towards NAC, or being hypersensitive to histamine; 5) involuntary admission, detainment or treatment according to the statutes defined in the Mental Health Act; 6) not able to speak or understand the Danish language; 7) for females: breastfeeding or planned pregnancy during the timeframe of the study.

Reason for study withdrawal: 1) serious adverse events suspected to be associated with the study treatment, based on an adverse rating scale; 2) severe suicidality during the trial, as judged by the investigator; 3) discontinuation of study treatment for seven or more consecutive days; 4) non-compliance with the defined study visits (visit within seven days prior or after the computer-generated date); 4) involuntary admission, detainment or treatment according to the statutes defined in the Mental Health Act; 5) withdrawal of consent; 6) for female participants: pregnancy or discontinuation of effective contraception.

Assessments and outcome measures

The primary outcome was the mean change from baseline up till week 20 in depressive symptoms as measured by the MADRS. The secondary outcomes were mean change in MADRS score from baseline up till week 24, as well as mean change on Bech-Rafaelsen Melancholia Scale (MES) (Bech, 2002a), Young Mania Rating Scale (YMRS) (Young et al., 1978), the WHO-Five Well-being Index (WHO-5) (Staehr Johansen, 1998), the Global Assessment of Functioning scale (GAF-F), the Global Assessment of Symptoms scale (GAF-S) (Jones et al., 1995) and the Clinical Global Impression-Severity Scale (CGI-S)) (Guy et al., 1976) at week 20 and up till week 24. The secondary outcomes included also proportions of responders (response defined as reduction of MADRS score ≥ 50% compared with baseline) and remitters (remission defined as MADRS score < 10 (Hawley et al., 2002) at week 20 and week 24.

The various rating scales were applied at weeks 0, 2, 10, 20 and 24. Additionally change in TAU during the trial, lifestyle behaviour (smoking, alcohol, substance use and BMI index) and treatment emergent adverse events or reactions were assessed and analysed. Adverse events and serious adverse events were reported by the study participants or healthcare professionals and documented at each study visit. Adherence was examined by counting all returned pills from each individual and thereby calculating days treated.

Statistical analysis

Descriptive analyses including mean and SD or number and percentage reported and simple tests such as independent sample t-tests, chi-square or Fisher's exact test were conducted on baseline variables to check for balance between the study groups in demographic data and other important participant characteristics. In all analyses participants were divided according to randomization.

The analysis of the primary outcome measure was based on all randomized study participants Intention-To-Treat (ITT)-population and by using the Linear Mixed Effects Repeated Measures (LMERM) analyses (Laird and Ware, 1982; Mallinckrodt et al.). A Generalized Estimating Equation (GEE) (Feng et al., 2001) approach with unstructured covariance pattern was implemented to account for within patients autocorrelation across study visits. The LMERM-analysis included the fixed categorical *study treatment, visits* and *visit by study treatment group two-way interaction* (intervention impact). The two-way interaction of visits and study treatment group examines between groups baseline adjusted mean differences at each post baseline visit, and hence is used estimate intervention impact. We first examined the overall intervention impact (i.e. examining overall effect of visits and study treatment group two-way interactions at all post baseline visits) and then examined MADRS at week 20 as a linear contrast of the implemented LMERM, as this was the a priori defined primary outcome measure. Similar approach was used for all other continuous secondary outcomes. Logistic regression models (with allocation group as the only independent variable entered) were applied to estimate Odds Ratios (OR) for dichotomised variables such as response and remission based on participants completing 20 weeks intervention period. Side effects were analysed by using Fisher's exact test. The Wilcoxon rank-sum test was used for comparing adherence and medical changes comparison between NAC and placebo.

All tests of treatment effects were conducted using a two-sided alpha level of 0.05 and 95% confidence intervals (CI) were reported. The sample size estimation was based on prior data from a similar clinical trial showing mean (SD) in MADRS score (from baseline to end of the study (week 24)) was decreased by around 10.0 ± 4.3 and increased by around 0.9 ± 2.6 (in respectively the NAC and the placebo group (Berk et al., 2008), and with an aim of being able to detect a potential baseline-adjusted between-group mean difference of least 8 ± 10.0 at week 20, with 80% power and a two-sided alpha-level of 0.05, 80 people were needed, allowing for a 30% dropout rate. All

data analyses were performed by the statistical software program STATA IC, version 15 (StataCorp., 2017), and in accordance with the International Conference on Harmonization E9 statistical principles .

RESULTS

Sample characteristics and participant disposition

A total of 192 patients were screened over a 2-year inclusion period (November 2014 until October 2016), with 80 patients out of these being randomized, equally distributed over time. A total of 53 people completed the intervention period (week 20); 26 in the NAC group and 27 in the placebo group (33.8% study withdrawals). Fifty-two participants completed the follow-up visit (24 weeks) (35.0% study withdrawals) with 26 participants in both groups. The detailed participant disposition is displayed in the CONSORT flow diagram (figure 1). Participant characteristics except from outcome measures are shown in table 1.

Figure 1

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Table 1:

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Except from two subjects in the NAC group, all participants received psychopharmacological treatment at baseline, most frequently antipsychotics, antidepressants and/or mood stabilizers, as shown in table 2. The mean number of psychotropic drugs prescribed at baseline was 3.8 (1.7), and there was a mean of 6.4 (2.9) psychopharmacological changes during the intervention period (increases, reductions, additions or/and discontinuations of treatment) with no differences between study groups (p=0.12) (data not shown).

Table 2:

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Efficacy of study treatment on the primary outcome

The LMERM-analysis showed no between-groups differences on mean change in MADRS scores (baseline adjusted) over the treatment phase (Intervention impact) ((NAC vs. placebo) 0.5; 95% CI:

-7.0, 5.9, p= 0.88) (table 3). The mean decline of the change in MADRS score from baseline to end of treatment (baseline unadjusted) was 13.8 points in participants allocated to NAC treatment as compared to 13.2 points in the placebo treated group. In addition, the overall intervention impact (i.e. testing all treatment by visits two-way interactions (simultaneously) showed no significant between group differences (p=0.53). The mean MADRS score at each visit between the two study groups are illustrated in figure 2. Results from the between-group comparisons from each study visit are shown in table 3.

Figure 2:

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Table 3:

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Follow-up (week 24) visit

As for the primary outcome analysis, the LMERM-analysis showed no between-group difference in mean change (baseline adjusted) from baseline to week 24 was seen (p=0.15, 95% CI: -11.6, 1.8), but the intervention impact was decreased to -4.9 (table 3). The mean MADRS score from week 20 to week 24 revealed a lower MADRS score in participants receiving NAC treatment compared to the placebo group corresponding to a decline in the mean MADRS score during these four weeks by 2.8 points in the NAC group and an increase by 1.3 points in the placebo group (table 3).

Efficacy of study treatment on secondary outcomes

The response rate from baseline to week 20 was 44.4% in the group receiving NAC treatment and 55.6% in the placebo group ((NAC vs. placebo) OR 0.69, 95% CI: 0.2, 2.0, p=0.49). In the group allocated to NAC treatment 47.8% achieved remission as compared to 52.17% in the group allocated to placebo ((NAC vs. placebo) OR 1.09, 95% CI: 0.4, 3.2, p=0.88).

After the extended four weeks blinded washout period, the response rate was 58.1% in the group allocated to NAC treatment group as compared to 42.0% in the group allocated to placebo

treatment ((NAC vs. placebo) OR 2.25, 95% CI: 0.7, 7.0, p=0.16), and for remission, a trend was found with 65.2% experiencing remission in the group allocated to NAC as compared to 34.8% in the group allocated to placebo ((NAC vs. placebo) OR 0.33, 95% CI: 0.1, 1.0, p=0.05).
There were no significant differences in change in any of the other secondary outcomes between treatment groups, except a lower YMRS in the group allocated to NAC at week 20 ($p < 0.03$) (table 3).

Safety and tolerability

In total, 66 adverse events were observed during the study (NAC group: n=39, placebo: n=27), with no between-group differences (OR 1.4, 95% CI: 0.8, 2.5, p=0.23) (Table 4). The most common adverse events were headache, diarrhoea, nausea and dizziness. Overall, 18 serious adverse events occurred during the study (NAC: n=10, placebo: n=8), with no difference between study groups (OR 2.0, 95% CI: 0.7, 6.1, p=0.20). These included five medical hospital admissions because of anaemia, influenza, pneumonia or alcohol intoxication, and the remainder were due to increased suicidal ideation (n=3) and psychiatric hospital admissions (n=10).

Table 4:

INSERT APPROXIMATELY HERE

Posthoc analyses

Based on the primary outcome, we explored whether the result would change, if only the more sensitive six-item subscale of MADRS ($MADRS_6$) (Bech, 2002b) was used. The $MADRS_6$ putatively covers the core symptoms of depression (Apparent and reported sadness, inner tension, lassitude, inability to feel, and pessimistic thoughts). However, a LMERM-analysis showed no between-group differences on the $MADRS_6$ ($p = 0.94$).
The psychopharmacological changes during the study were considered to possibly impact on outcome results, why changes in psychopharmacological treatment (from one drug to another or discontinuation) was included as a confounder in the LMERM-analysis, but did not change the primary result (95% CI: -7.0, 6.0, $p = 0.89$). Adherence to study medication during the trial was

evaluated based on calculation of all the returned pills from each participant and no between-group difference was found.

DISCUSSION

In this 20-week trial, we were unable to show any difference on the primary measure, i.e. the mean change on the MADRS score, between participants treated with adjunctive NAC and participants treated with adjunctive placebo. Likewise, at week 24, i.e. four weeks after study medication discontinuation, no statistically significant between-group difference in the mean change on the MADRS score was seen. However, at week 24, there was a trend towards a higher remission rate in the NAC group (65.2%) as compared to the placebo group (34.8%) (p=0.05), reflecting a worsening in the MADRS score in the placebo group between week 20 and week 24. The four-week washout phase was incorporated into the study design to evaluate whether a potential superior effect of NAC as compared to placebo would be maintained after drug discontinuation. However, given that no superiority of NAC could be demonstrated during the treatment phase, any interpretation of this secondary finding besides considering it as a type one error is speculative, like e.g. that the relatively large placebo effect (see below) diluted a minor effect of NAC, an effect that became observable after the placebo effect disappeared in both groups.

Two out of the three previously published efficacy studies of NAC treatment in BD were positive and one was negative (Berk et al., 2008; Berk et al., 2011; Berk et al., 2012). The eligibility criteria of these studies were similar to the current study. Compared to the previous studies, in the present study a higher dose of NAC treatment was administered, changes in psychopharmacological treatment prior to inclusion were allowed and participants were more severely depressed. These factors might have impacted the outcome of the study. Additionally, the use of a pre-defined baseline severity for inclusion may have increased the risk of inflated baseline scores, resulting in apparent increased treatment response in both groups, thereby minimizing potential between-group differences (Fournier et al., 2010; Landin et al., 2000).

Our study investigated adjunctive treatment to TAU to reflect real world clinical practice. In studies investigating the efficacy of adjunctive treatment it is difficult to detect a difference

between study groups, since it has to generate efficacy in addition to an already established treatment. This is especially the case if the established treatment is initiated shortly prior to the study or if it can be changed during the trial by the clinician, as placebo treated patients not experiencing any improvement most likely will undergo changes in pharmacological or psychotherapeutic treatment to improve their condition. On the other hand, patients in the current study were experiencing an acute episode of depression and a pure placebo add-on treatment arm, without the possibility of adjusting background treatment for the study duration of 24 weeks was not judged ethically acceptable and would most likely also have resulted in more drop-outs. However, if NAC treatment was effective, we could have expected fewer alternations in the NAC group than in the placebo group during the study; such a difference was not observed, thus lending further support to the null hypothesis of no true antidepressant effect of NAC in bipolar depression. The anticipated NAC effect used in sample size calculation was optimistic and in addition dropout rate was higher than expected. As such the study might have been underpowered and this could be another reason why treatment effect was not statistical significant.

Adherence to medication in patients diagnosed with bipolar disorder has been shown to be low with a median medication possession ratio of approximately fifty percent (REF)(Berk and Berk, 2003; Greene et al., 2018). In the current study we utilized pill counts of returned packages of medication to calculate an adherence rate, showing no significant difference between study groups. Other methods to estimate adherence have been proposed such as electronic pill bottles, although are rarely used(Vrijens and Urquhart, 2014).
In the placebo group, we found a mean reduction in MADRS scores of 55.6% over 20 weeks, which is higher than previously found by Berk et al, who observed a 21.0% reduction over 20 weeks in the placebo group (Berk et al., 2008). In general, studies with high placebo response rates are likely to result in absence of an efficacy signal from any treatment, which should be taken into consideration in the interpretation of our negative results.

The main strengths of the current study were the use of a randomized double-blind placebo-controlled design and the fact that the planned study sample was achieved. Also, the allowance of

medication changes throughout the study period increased the generalizability and clinical utility of the results.

In conclusion, despite a sufficient sample size adjunctive NAC treatment showed no superiority to placebo in terms of efficacy in any of the psychometric outcome measures from baseline to week 20 except from the YMRS, which was clinically insignificant. The findings emphasize the challenge of interpreting negative findings of clinical trials when placebo response rates are high. Also, the fluctuations of mood over time in BD calls for development of more sensitive outcome measures. Currently, studies have shown effect of NAC in the treatment of depression in patients diagnosed with bipolar disorder(Berk et al., 2008; Berk et al., 2011). The treatment is generally well-tolerated and the current treatment armamentarium for treating depression in patient diagnosed with bipolar disorder is small. As a result, we would suggest further studies are needed to clarify whether NAC has an effect against depression in this population and if the medication should be recommended as a possible treatment option.

AUTHOR STATEMENT CONTRIBUTORS

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Journal of Affective Disorders.*

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DECLARATION OF INTEREST

PK Ellegaard has received speaking fee from Lundbeck. **RW Licht** has received research grant from Glaxo Smith Kline, honoraria for lecturing from Pfizer, Glaxo Smith Kline, Eli Lilly, Astra-Zeneca, Bristol-Myers Squibb, Janssen Cilag, Lundbeck, Otsuka, Servier and honoraria from advisory board activity from Glaxo Smith Kline, Eli Lilly, Astra-Zeneca, Bristol-Myers Squibb, Janssen Cilag, and Sunovion. **RE Nielsen** has received research grants from H. Lundbeck and Otsuka Pharmaceuticals for clinical trials, received speaking fees from Bristol-Myers Squibb, Astra Zeneca, Janssen & Cilag, Lundbeck, Servier, Otsuka Pharmaceuticals, and Eli Lilly and has acted as advisor to Astra Zeneca, Eli Lilly, Lundbeck, Otsuka Pharmaceuticals, Takeda, and Medivir. **OM Dean** has received grant support from the Brain and Behavior Foundation, Simons Autism Foundation, Stanley Medical Research Institute, Deakin University, Lilly, NHMRC and ASBDD/Servier. She has also received in kind support from BioMedica Nutraceuticals, NutritionCare and Bioceuticals. **M Berk** has received Grant/Research Support from the NIH, Cooperative Research Centre, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, MBF, NHMRC, Beyond Blue, Rotary Health, Geelong Medical Research Foundation, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Meat and Livestock Board, Organon, Novartis, Mayne Pharma, Servier, Woolworths, Avant and the Harry Windsor Foundation, has been a speaker for Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck, Merck, Pfizer, Sanofi Synthelabo, Servier, Solvay and Wyeth, and served as a consultant to Allergan, Astra Zeneca, Bioadvantex, Bionomics, Collaborative Medicinal Development, Eli Lilly, Grunbiotics, Glaxo SmithKline, Janssen Cilag, LivaNova, Lundbeck, Merck, Mylan, Otsuka, Pfizer and Servier. **HE Poulsen**, **M Mohebbi** and **CT Nielsen** has no conflicts of interest to declare.

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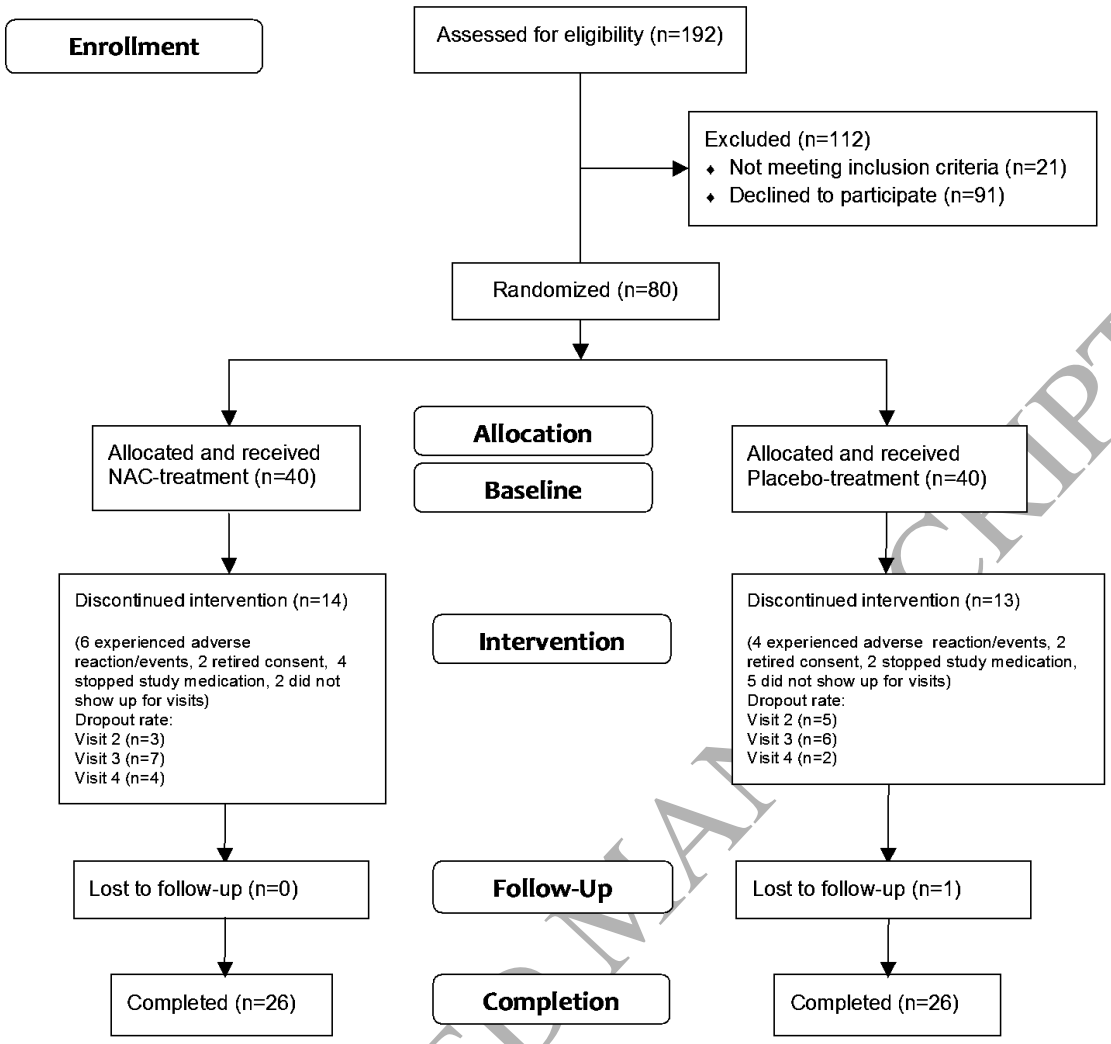
TABLES AND FIGURES

Figure 1: CONSORT 2010 Flow Diagram. Flow diagram of the progress through the phases of the randomized trial from enrolment, intervention allocation, follow-up to completion.



Abbreviation: NAC: N-acetylcysteine.

Table 1: Descriptive characteristics of study participants at baseline

Measurements	Overall (n=80)	NAC (n=40)	Placebo (n=40)
Mean Age (SD) (years)	43.4 (10.1) [a]	43.7 (10.0)	43.0 (10.2)
Gender (no. of females, %)	47 (59.0%) [b]	26 (65.0%)	21 (52.5%)
Number of outpatients (%)	60 (75.0%) [b]	31 (77.5%)	29 (72.5%)
Diagnosis: Bipolar type I (n, (%))	21 (26.0%) [b]	9 (22.5%)	12 (30.0%)
Diagnosis: Bipolar type II (n, (%))	59 (74.0%) [b]	31 (77.5%)	28 (70.0%)
Mean age at diagnosis (SD) (years)	37.9 (9.8) [a]	38.4 (9.3)	37.4 (10.4)
Mean age at onset of illness (SD) (years)	19.9 (9.8) [a]	20.0 (10.5)	19.9 (9.3)
No. of depressive episodes > 10 (n, (%))	59 (83.1%) [b]	28 (82.4%)	31 (83.8%)
No. of hypomanic/manic episodes > 10 (n, (%))	46 (66.7%) [b]	21 (65.6%)	25 (67.6%)
No. of psychiatric hospitalizations (mean (SD))	4.2 (7.8) [a]	3.0 (4.8)	5.4 (9.9)
No. of patients with previous suicidal attempts (n, (%))	32 (40.0%) [b]	13 (32.5%)	19 (47.5%)
Previously ECT-treatment (n, (%))	17 (21.3%) [b]	7 (17.5%)	10 (25.0%)
Mean ECT-treatments (SD)	18.7 (12.2) [a]	17.6 (7.6)	19.5 (15.0)
No. of cigarette smokers (n, (%))	47 (59.0%) [b]	23 (57.5%)	24 (60.0%)
Mean cigarettes pr. day (SD)	19.6 (8.4) [a]	20.8 (9.9)	18.4 (6.7)
No. who drinks alcohol (n, (%))	42 (52.5%) [b]	22 (55.0%)	20 (50.0%)
Mean alcohol units[c] weekly (SD)	14.1 (25.2) [a]	11.5 (16.4)	16.9 (32.5)
Drug intake prior to inclusion (n, (%))	8 (10.0%) [b]	4 (10.3%)	4 (10.3%)
Mean BMI (SD)	28.3 (6.0) [a]	28.3 (6.9)	28.2 (5.1)
Mental Health Service Esbjerg (n, (%))	34 (42.5%) [b]	17 (50.0%)	17 (50.0%)
Mental Health Service Vejle (n, (%))	30 (37.5%) [b]	14 (46.7%)	16 (53.3%)
Mental Health Service Aalborg (n, (%))	16 (20.0%) [b]	9 (56.3%)	7 (43.7%)

Abbreviations and notes: a: t-test, b: Parsons's X^2, c: one unit of alcohol in Demark is defined as 15 millilitres (12 grams) of pure alcohol; BMI: Body Mass Index. Data are expressed as mean (Standard Deviation) or as number and percentage, depending on the type of data. No significant difference between treatment groups was found based on linear regression analyses. NAC: N-acetylcysteine.

Table 2: Pharmacological treatment at baseline

	Baseline (n (%))		
Psychopharmacological classification	**Overall**	**NAC**	**Placebo**
Antidepressants	42 (52.5%)	21 (52.5%)	21 (52.5%)
Antipsychotics	59 (73.8%)	29 (72.5%)	30 (75.0%)
Quetiapine	49 (61.3%)	25 (62.5%)	24 (60.0%)
Others	17 (21.3%)	08 (20.0%)	09 (22.5%)
Mood stabilizers	42 (52.5%)	21 (52.5%)	21 (52.5%)
Valproate	13 (16.3%)	05 (12.5%)	08 (20.0%)
Lamotrigine	35 (43.8%)	20 (50.0%)	15 (37.5%)
Others	08 (10.0%)	06 (15.0%)	02 (05.0%)
Lithium	27 (33.8%)	11 (27.5%)	16 (40.0%)
Benzodiazepines	10 (12.5%)	06 (15.0%)	04 (10.0%)
Hypnotics	13 (16.3%)	08 (20.0%)	05 (12.5%)
No psychopharmacy	02 (02.5%)	02 (05.0%)	00 (00.0%)
Psychotherapy	63 (78.8%)	32 (80.0%)	31 (77.5%)

Abbreviations and notes: Data are expressed as number and percentage using a two-way table. Based on Parsons's chi-squared tests no significant difference between treatment groups was found. NAC: N-acetylcysteine.

Table 3: Primary (change in MADRS score from baseline to 20 weeks) and secondary outcome measures comparing NAC group and placebo group

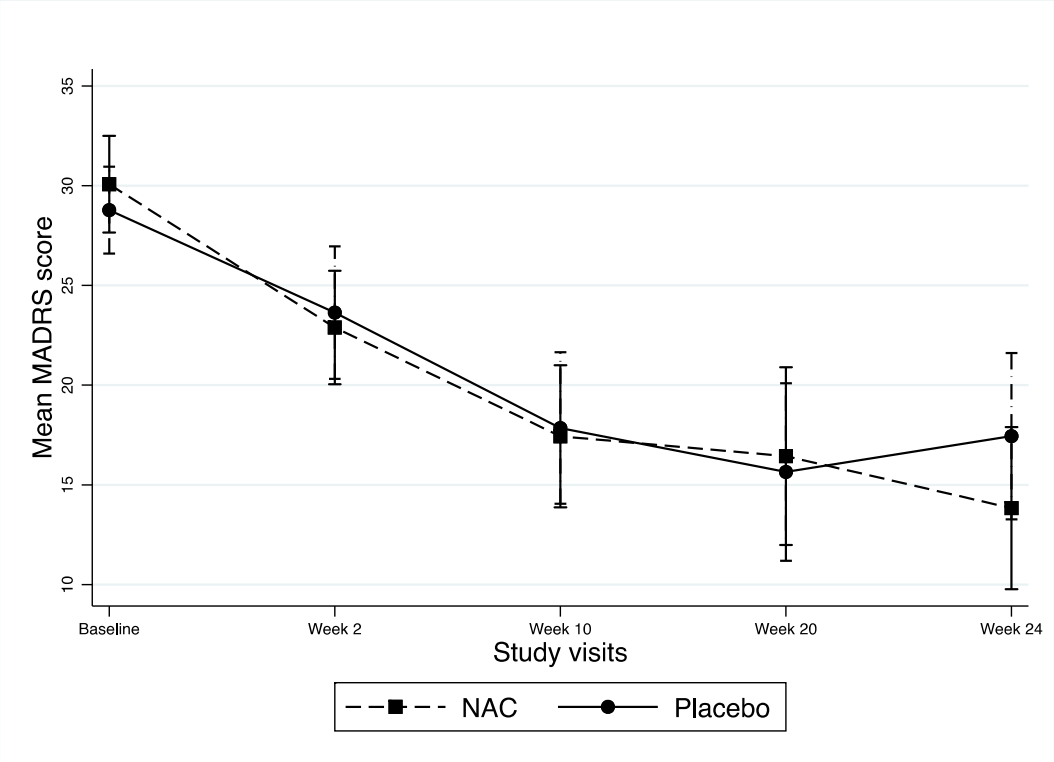
		NAC	Placebo	Baseline adjusted models		
		Mean (SD)		p-value	Intervention impact [a]	95% CI
MADRS	Baseline	30.1 (7.9)	28.8 (7.1)			
	2 weeks	23.0 (1.5)	23.9 (1.7)	0.34	-2.0	-6.2, 2.1
	10 weeks	18.1 (1.9)	17.7 (2.0)	0.54	-1.7	-7.2, 3.7
	20 weeks	16.3 (2.3)	15.6 (2.4)	0.88	-0.5	-7.0, 5.9
	24 weeks	13.5 (2.1)	16.8 (2.1)	0.15	-4.9	-11.6, 1.8
	Overall			0.53		
MES	Baseline	21.5 (5.5)	21.1 (4.6)			
	2 weeks	15.8 (1.1)	16.4 (1.2)	0.20	-1.9	-4.7, 1.0
	10 weeks	12.4 (1.3)	12.2 (1.4)	0.46	-1.4	-5.1, 2.3
	20 weeks	11.7 (1.6)	19.3 (1.5)	0.93	0.2	-4.4, 4.0
	24 weeks	9.2 (1.5)	11.6 (1.4)	0.07	-4.0	-8.3, 0.3
	Overall			0.17		
YMRS	Baseline	2.3 (2.8)	1.9 (2.6)			
	2 weeks	1.6 (0.3)	2.5 (0.6)	0.12	-1.3	-3.0, 0.3
	10 weeks	2.1 (0.8)	2.9 (0.7)	0.26	-1.1	-3.0, 0.8
	20 weeks	1.2 (0.6)	2.5 (0.7)	0.03*	-1.6	-3.1, -0.2
	24 weeks	1.3 (0.5)	1.2 (0.5)	0.48	-0.5	-1.9, 1.0
	Overall			0.05*		
WHO	Baseline	21.7 (12.6)	20.9 (16.1)			
	2 weeks	35.4 (3.7)	32.0 (3.5)	0.59	2.7	-7.1, 12.4
	10 weeks	40.1 (4.6)	45.0 (4.0)	0.43	-5.0	-17.4, 7.4
	20 weeks	48.3 (4.8)	42.7 (5.7)	0.69	2.9	-11.1, 16.8
	24 weeks	51.1 (5.2)	42.3 (4.1)	0.30	7.2	-6.5, 20.9
	Overall			0.41		
GAF-F	Baseline	48.7 (9.3)	49.7 (8.9)			
	2 weeks	50.9 (1.5)	51.3 (1.6)	0.50	0.7	-1.4, 2.9
	10 weeks	54.7 (2.0)	54.2 (2.0)	0.42	2.0	-3.0, 6.9
	20 weeks	55.5 (2.5)	55.1 (2.5)	0.57	1.9	-4.6, 8.3
	24 weeks	59.4 (2.5)	60.3 (2.8)	0.75	1.2	-6.2, 8.6
	Overall			0.94		
GAF-S	Baseline	50.6 (6.5)	51.0 (6.7)			
	2 weeks	53.8 (1.2)	52.9 (1.3)	0.32	1.4	-1.4, 4.2
	10 weeks	56.7 (1.8)	55.7 (1.8)	0.44	1.7	-2.7, 6.1
	20 weeks	59.2 (2.1)	59.4 (2.0)	0.80	0.7	-4.6, 5.9
	24 weeks	61.9 (2.1)	61.8 (2.0)	0.61	1.5	-4.4, 7.4
	Overall			0.84		
CGI	Baseline	4.3 (0.6)	4.3 (0.5)			
	2 weeks	4.1 (0.1)	4.2 (0.1)	0.67	-0.1	-0.3, 0.2
	10 weeks	3.9 (0.1)	3.8 (0.1)	0.81	0.0	-0.3, 0.4
	20 weeks	3.5 (0.2)	3.6 (0.1)	0.70	-0.1	-0.5, 0.4

	24 weeks	3.4 (0.2)	3.4 (0.1)	0.81	-0.1	-0.5, 0.4
	Overall			0.94		

Abbreviations and notes: MADRS: Montgomery-Asberg Depression Rating Scale score; MES: Bech-Rafaelsen Melancholia Scale; NAC: N-acetylcysteine; WHO-5: World Health Organization Well-Being Index; GAF-S: The Global Assessment of Symptoms scale; GAF-F: The Global Assessment of Functioning scale; YMRS: Young Mania Rating Scale (YMRS)), CGI-S: The Clinical Global Impression-

Severity scale (CGI-I). A high score in MADRS, MES, CGI-S-S and YMRS represents a severe disease degree/level/rate, while a high score in WHO-5, GAF-F and GAF-S represents a low disease degree/level/rate. a: Baseline adjusted mean differences model between NAC and placebo group, and NAC group as reference category). *Significant at p = 0.05

Figure 2: Margin plots of MADRS score at each visit divided by treatment groups



Abbreviations and notes: MADRS: Montgomery-Asberg Depression Rating Scale score; NAC: N-acetylcysteine. Testing overall study treatment by visits two-way interactions (intervention impact across all post-baseline visits)

Table 4:

Table 4: Adverse Reactions and Serious Adverse Events reported during the study

Adverse Reactions	**NAC**	**Placebo**	**NAC vs. Placebo** (p-values)
Headache	4	3	>0.99
Diarrhoea	6	5	>0.99
Nausea	8	0	0.005**
Dizziness	3	4	>0.99
Reflux	4	0	0.12
Tremor	2	4	0.68
Dry mouth	1	3	0.62
Skin rash	2	1	>0.99
Heartburn	2	0	0.50
Increased sweat	2	2	>0.99
Obstipation	2	2	>0.99
Blurred vision	1	1	>0.99
Stomach pains	1	1	>0.99
Tinnitus	1	1	>0.99
Adverse Reactions in total (n=66)	39	27	0.23
Serious Adverse Events	**NAC**	**Placebo**	**NAC vs. Placebo** (p-value)
Psychiatric hospital admission	5	5	>0.99
Medical hospital admission	4	1	0.40
Acute increase suicidal	1	2	>0.99
Serious Adverse Events in total (n=18)	10	8	0.20

Abbreviations: The p-values are based on Fisher's exact probability test. *Significant at p = 0.005.

NAC: N-acetylcysteine.